THE EMBRYOLOGY OF THE WHITEFISH *COREGONUS CLUPEAFORMIS*, (MITCHILL)

PART II. ORGANOGENESIS

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FOREWORD

In the first paper of this series,[1] the early embryology of the whitefish from fertilization up through the closure of the blastopore (Stage 128, O. S. U. series) was described. The later stage marked the completion of the first third of the incubation period in terms of thermal units, although the embryo had been incubated only one-sixth the number of days usually required by this species before hatching in lake water of winter temperatures. The embryo was about 3 mm. long, and it lay without torsion and clearly outlined over the curvature of the yolk. In its anterior end, the three primary brain lobes were present and the neural keel of the head region lay deeply imbedded in the yolk. The optic primordia were prominent, lateral to the forebrain and were attached to the latter by broad optic stalks. Neither the brain nor the optic anlagen possessed cavities in this stage and hence have not yet acquired the vesicular condition. A thickened ectodermal plate lay on each side of the neural keel lengthwise. This sensory plate possessed an indistinct sensory furrow. The notochord had extended its full length forward to end beneath the midbrain. Eleven pairs of somites were present, with the most anterior one lying at the level of the hind brain. The entoderm was described as being thin and unfolded throughout its length. Kupffer's vesicle was seen here in its stage of maximum size.

From this condition as a point of departure, the present paper describes the general embryology and development of the whitefish up through Stage 400 of this series, which marks the completion of the first half of the incubation period.

Stage 144. O. S. U. Series.—(Incubation period, 24 days, 20 hours; 112.5 T. U.; fourteen pairs of somites.) The embryo lies as in the

[1]The Embryology of the Whitefish. Part I. Early Embryology. Ohio Jour. Sci., Vol. XXXIV, No. 5, 1934.

previous stage prominently elevated above the yolk surface, without torsion. It now extends about one-half the distance around the curvature of the yolk. Marked cephalization is expressed in the prominence of the three primary brain lobes and of the optic vesicles. The latter are now well developed sac-like structures whose walls are composed of a single layer of columnar cells. The cavity within is confluent with a small one within the brain stem which appears in this stage for the first time as the result of continued delamination and final separation of the cells of the neural keel in that region. This cavity is the third ventricle. Elsewhere in the brain region, the cells are not definitely separated, but their nuclei are drawn out to the sides of the keel, and their inner ends are interlocked. Their nuclei thus form two distinct rows, one on each side of the mid-line. The central portion of the sensory plate has become invaginated at the level of the hind brain to form a thick-walled auditory pit, with a small central canal. A thickened ectodermal plate lays anterior and continuous with it to form the pre-auditory sensory plate. In the posterior end of the notochord, the cells are definitely wedge-shaped and interlocked. Their nuclei are migrating laterally toward the periphery, preparatory to vacuolation of the notochord. The entodermal sheet retains its unfolded condition forward to the branchial region at the level of the first anterior somite. Anterior to this level it becomes folded on itself dorso-laterally to form a conspicuous branchial fold on either side. However, the floor of the pharynx is incomplete in the mid-line. Just anterior to the auditory pit, this projects distally as a pouch to meet a plate of surface ectoderm on the side of the head to mark the location of the first branchial cleft. Just anterior to this, the hyomandibular pouch extends more vertically, but does not reach the surface ectoderm. From this point forward the branchial folds flatten out and the pharynx ends just beyond the end of the notochord. Lateral to the branchial folds on either side lies a sheet of splanchnic mesoderm which is folded inward to enclose minute paired pericardial cavities. The cells on the mesial border of these folds are somewhat elongated. Inward from them lie clusters of cells derived from intermediate mesoderm, which are destined to form the endocardial lining of the heart.

Stage 160. O. S. U. Series.—(Incubation period, 27 days, 12 hours; 115.1 T. U.; 21 pairs of somites; total length of embryo, 3.5 mm.)

The embryo has undergone an orderly increase in length and in the number of paired somites during the two and a half days of incubation since the previously described stage. The brain has increased in size, with thicker walls. The third ventricle is more pronounced in the surface view, and now extends as a narrow cavity above the floor of the prosencephalon throughout its length. The optic vesicles likewise are larger than in the previous stage. They have now become laterally compressed and invaginated to form a two-layered optic cup. The elongated columnar cells which line the cup to form the thickened retinal layer are now separated mesially from the thinner peripheral layer of cells by a narrow cavity, confluent with that of the optic stalk and third ventricle. Ventrally, the optic cup is indented by the choroid

fissure, which is formed as the result of the invagination of the cup, and which persists until after the hatching stage. The small area of surface ectoderm which covers the center of the optic cup is thickened at this stage to form the primordium of the lens. A slight invagination of a thickened ectodermal placode on each side of the mid-line, on the antero-ventral surface of the head marks out for the first time the location of the nasal pits. The auditory pit, described in the previous stage, has sunken further beneath the surface and now forms the auditory vesicle, whose thick walls are composed of a single row of columnar cells concentrically arranged. Its mesial surface comes to lie in close proximity to the sides of the hind brain. The branchial folds, first described in the previous stage, are more conspicuous in cross-sections than before. The hyomandibular pouch now is in contact with a thickened ectodermal plate on the outer surface of the head. The first branchial pouch immediately behind it clearly extends to the surface ectoderm, laterally beneath the anterior portion of the auditory vesicle. Cross-sections at these levels closely approximate the conditions shown in Figures 56 and 59, Plate XVI—Swaen *et* Brachet—1900. Anterior to the hyomandibular pouch the entoderm has folded on itself to complete the floor of the pharynx in the mid-line by a thin strand of cells. The pharynx ends blindly as before.

The endocardial masses from each side slightly in advance of the level of the auditory vesicle have met each other in the mid-line beneath the enclosed portion of the pharynx, forming a single, loosely connected endocardial mass. The folded layers of splanchnic mesoderm bounding the pericardia have accompanied the endocardial masses in their migration inward and now lie ventro-lateral beneath the pharynx. See Fig. 16, Pl. XVII, Swaen et Brachet, v. 16.

There are apparently 21 pairs of somites present. In the anterior somites particularly, the cells on the ventro-mesial border are assuming the mesenchymal characteristics of the sclerotome. The latter lies adjacent to the notochord.

In Stage 112, the intermediate mesoderm in the region of the anterior somites was described as a single line of cells which at that time lies between the somite and the lateral mesoderm. Now these cells have increased in number, have migrated ventrally and form a wedge-shaped mass ventro-lateral to the sclerotome and dorsal to the entoderm. These masses of intermediate mesoderm will later complete their migration from each side and occupy the space in the mid-line beneath the subnotochordal rod, there to give rise to the blood and definitive vessels in this region.

In Teleosts, the intermediate mesoderm is not segmented, and hence the primitive excretory organ does not form as a series of pronephric tubules but rather as a single pronephric chamber.

At the level of the 4th, 5th, and 6th somites, a secondary plate of intermediate mesoderm, which has not migrated as above but has retained its primitive position on the mesial face of the lateral mesoderm, gradually becomes differentiated from the latter to form the pronephric chamber. This differentiation appears first as a thickening of this plate. Its ventral mesial border becomes folded sharply to grade imperceptibly

into the splanchnic layer of the lateral mesoderm. Later, when the cavity of the splanchnocoele forms, it will penetrate between the folded cell layers of this plate to form the lumen of the pronephric chamber. From the level of the 7th somite backwards, the cells of the secondary intermediate plate become constricted off from the mesial face of the lateral mesoderm to form a solid cord of cells which appears as a rosette in cross-sections. This line of cells becomes the pronephric duct, after it acquires a lumen, and extends to the anus. At the present stage, number 160, the first steps in the differentiation of the pronephric chamber and duct have occurred. At the level of the 4th, 5th, and 6th somites, the primitive plate of intermediate mesoderm is thickened and its ventral mesial border is drawn slightly downward and inward. Its cells are compressed, and no cavity is present. The line of cells marking off the pronephric duct can be traced in these sections from somites 7 to 12. The lumen of this duct has likewise not yet formed. The development follows the description of Swaen and Brachet (1900) and is slightly less advanced than is figured by them in Figures 17, 18, and 19, of Plate XII.

In the angle on each side of the body opposite the first anterior somites there is a longitudinal furrow paved with columnar epithelial cells. At the intervals between somites, these cells form a conspicuous cluster. Anterior to the first somite this thickening swings dorsally and approaches the auditory sac. Whether this line of cells represents an early stage in the development of the lateral line, or whether it is simply due to other and unrelated development of the surface ectoderm, the author is unable to determine at this point.

Stage 176. O. S. U. Series.—(Incubation period, 30 days, 4 hours; 117.8 T. U.; 24 somites.) The degree of development in this stage of the brain and sense organs is not significantly different than that described in the previous stage. The cells of the brain and nerve cord are now regularly disposed throughout the length of the body along the lateral margins and are separated by a narrow line throughout the dorsal portion. The canalis centralis extends the length of the nerve cord near its floor. In sagittal sections, a mass of head mesoderm is seen in the same relative position as that described in Stage 128. It is now larger and triangular in shape. The apex of the triangle is near the superficial ectoderm on the lateral margin of the head. In the branchial region, the auditory vesicle, branchial folds and endocardium have developed to a slightly more advanced stage but their relationships are essentially the same as in the previous description. The folds of the gut rapidly diminish in extent in the esophageal region, and merge into an unfolded sheet of entoderm at the level of the 2d. somite. A minute lumen appears between the entodermal cells in the mid-line opposite the middle of the 3d. somite, and continues for the length of almost five somites. Sclerotome formation is more advanced in the anterior somites but can be traced for at least 15 somites. Temporary cavities are present in the sclerotomal masses of the first five or six somites. The primary plates of intermediate mesoderm which are the primordia of the axial blood vessels retain the position described in Stage 160, ventro-lateral to the sclerotome and dorsal to the entoderm. These

wedge-shaped masses extend in toward the mid-line particularly between the somites. From the 6th to the 9th somite level a few cells have reached the mid-line and penetrate the cavity beneath the subnotochordal rod. This condition is less distinct at other levels.

The degree of development of the pronephric chamber is only slightly more advanced than described in the preceding stage. The primordium of the pronephric duct still lacks its lumen but can be traced from somites seven to eighteen.

The ectodermal thickenings along the sides of the body can be located in sections on the side of the trunk opposite the divisions between somites backward as far as the interval between the 7th and 8th somites.

Stage 192. O. S. U. Series.—(Incubation period, 32 days, 20 hours; 119.5 T. U.; approximately 28 somites.) The mid-brain is now divided into two distinct optic lobes separated by a conspicuous Sylvian aqueduct, and the hindbrain is differentiated into the metencephalon and myelencephalon. The walls of the brain stem in this area have become thickened, and the fourth ventricle appears for the first time with this stage. The optic stalk is more constricted than described in Stage 160. The conditions through the middle of the body are the same as described for the previous stage, with the following modifications. The intermediate cell masses still lie ventral to the sclerotome on either side but are composed of a larger number of cells and hence are more prominent. More cells than before have migrated from this mass into the mid-line beneath the subnotochordal rod. There are approximately twenty-eight somites present. The primordium of the pronephric duct may be traced from the seventh to the twenty-second somite.

The tail is distinctly raised above the yolk, and the tail fold is beginning to undercut it.

Stage 208. O. S. U. Series.—(Incubation period, 35 days, 12 hours; 123.14 T. U.; 32 somites.) The fore-brain is now differentiated into telencephalon and diencephalon. The infundibulum is present here in its initial stages of development as a slight thickening beneath the floor of the diencephalon, adjacent to the point of emergence of the optic stalk. The blind anterior end of the pharynx is immediately posterior to the infundibulum. Here both the upper and lower layers of the pharyngeal wall extend laterally and are in continuity with the surface ectoderm on the side of the head. Both the hyomandibular and first branchial pouches are definitely in contact with the slightly invaginated surface ectoderm. The first branchial pouch extends diagonally from just before the auditory pit, backward and downward beneath it. Midway beneath the auditory pit the pharynx rounds up sharply into the gut which possesses a lumen for a short distance. Posteriorly, the entoderm is a highly arched layer in the mid-ventral line whose lower edges have not yet fused, and which is flattened out progressively caudad. The endocardial primordium lies as a single mass of loosely connected cells beneath the pharynx backward to the level of the first branchial pouch. It is somewhat more sharply delineated from the epimyocardial folds than described in Stage 160. The epimyocardial folds likewise are more thickened in cross-section than in the former stage.

The pronephric ducts may be traced from somites seven to twenty-two as before. There are 32 somites present. Posterior to the last somite there extends a non-segmented mesodermal mass backward to the level of Kupffer's vesicle, which lies beneath the caudal end of the notochord.

Stage 224. O. S. U. Series.—(Incubation period, 38 days, 4 hours; 125.8 T. U.; total length of embryo, 4.2 mm.; 39 somites.—Reconstruction drawing, Plate III.)

The reconstruction drawing of this stage may be directly compared with that of Stage 128, Plate II, in the first paper of this series. It is seen that the embryo has increased more than one-third its former length in the interval between these two stages. By this time it has passed through a little more than one-fourth its total incubation period, in point of time (38 days out of a total of 134 days), and one-third of its incubation period in point of thermal units (126 T. U. out of a total of 345 at hatching). Its present length of 4.2 mm. is likewise about one-third of its hatching length, (12 mm.). It is of significance that in this first third of its incubation period, every major organ system and structure present at hatching with the exception of the paired pectoral fins has been definitely laid down. The embryo at this time is clearly outlined on the surface of the yolk. It extends without torsion over approximately one-half of that surface, and its anterior end is somewhat imbedded in it. The conspicuously large brain and sense organs, particularly the eyes, indicate a precocious development in that region.

The definitive brain lobes, including the infundibulum, are established and can be distinguished in the whole mount. The nasal pit is in the form of an elliptically invaginated sac, lined with columnar epithelium, on the antero-lateral angle of the forebrain. The prominent eye possesses a well-developed circular lens centrally placed, and the optic nerve is seen to extend forward from the mesial-ventral surface of the eye to enter the diencephalon at the anterior end of the infundibulum. The head is imbedded in the yolk up to the level of the lens. Extending over the yolk laterally from this point is a sheet of extra-embryonic somatopleure shown in the drawing as a lightly stippled band.

The brain has greatly increased in width, particularly in the region of the medulla oblongata, where it is much broader than deep. This widening process has brought about a great expansion of the fourth ventricle, which is very conspicuous from the dorsal view. Triangular in shape, its area is approximately equal to that of the remainder of the brain. The choroid plexus which covers its roof is seen in the drawing to be clearly separated from the underlying nervous tissue. Laterally, the auditory sac is a rounded invaginated vesicle of columnar cells closely applied to the sides of the medulla. The medulla posterior to the auditory sac rounds up rapidly into the tubular nerve cord which is well developed and prominent in this stage and continues to the tail region. There, the nerve cord fades out into a caudal mass which is losing its embryonic character.

The process of vacuolation of the cells of the notochord which began

in Stages 128 and 144 has progressed and now is evident throughout its length. The cells as a result of this process have been greatly enlarged and the notochord has been increased in diameter. The notochord acquired its maximum length in Stage 128. It extends forward to end beneath the medulla, approximately at the anterior end of the pharynx. The anterior end of the notochord from this time on appears to be a relatively fixed point, with respect to the floor of the medulla, but the relative positions of the anterior end of the pharynx and of the infundibulum become greatly altered as development proceeds.

The foregut terminates at a point posterior to the orbit, and in contact with the underlying surface of the head region. Neither in this stage nor subsequently is there any indication of the presence of a stomodaeum. The end of the foregut is some 100 microns behind the posterior end of the infundibulum, but in later stages, these two structures come in contact with each other.

The foregut is completely folded on itself. It arches broadly laterally, and extends to the surface ectoderm. The branchial furrow is essentially an infolded portion of thickened ectoderm in the angle on the side of the head. The hyomandibular pouch is in contact with this furrow but no cleft is formed. The first branchial pouch beneath the anterior portion of the auditory sac has broken through this furrow to form an open gill cleft, in which the pharyngeal wall is continuous with the surface ectoderm. The ectoderm lines the outer end of the cleft for a comparatively short distance, leaving the remainder of the cleft lined by entoderm. A few sections posterior to the first branchial cleft, the second branchial pouch appears for the first time and extends laterally to come into contact with the lateral ectodermal plate for a short distance.

The pharynx itself does not possess a lumen, but as it rounds up into the esophagus, just behind the second branchial pouch, a lumen appears in the future gastric region. The gut at this stage is in the form of a closed tube throughout its entire length, lined with columnar epithelium. Its lumen gradually diminishes in diameter posteriorly. At the level of the 36th and 37th somites, the tubular gut terminates in a loosely organized strand of entodermal cells which marks the location of the future anus. Beyond that point, the entoderm continues as a solid strand of cells, the post-anal gut. This soon loses its identity, along with the notochord in the remains of the primitive streak. The post-anal gut overarches Kupffer's vesicle which now lacks a cellular floor above the yolk.

The heart has developed progressively beyond the condition described in the previous stage. The endocardium is clearly separated from the lateral epi-myocardial layers, and forms a single straight tube which extends beneath the anterior end of the pharynx from its anterior end to the level of the first branchial cleft. Here it bifurcates into two vitelline veins which spread laterally over the surface of the yolk. Paired dorsal aortae appear above the hymandibular pouch and extend backward to fuse in the midline beneath the notochord just beyond the level of the second branchial pouch. The single dorsal aorta may be traced from this point backward to the level of the eighth somite.

More caudally, its outline is lost in the undifferentiated intermediate cell mass in the median line from which it arises.

In order to avoid complexity no attempt was made to place either the somites or the pronephric tubules and ducts on the reconstruction drawing of this stage. The somites number 39 pairs, and extend from just posterior to the auditory sac, as before, throughout the body, to a point opposite the anterior end of Kupffer's vesicle. Behind the last somite, the mesoderm continues as an undivided mesodermal mass, which terminates in the primitive streak. The myotomes of the first anterior six somites possess myofibrils. The pronephric chamber possesses a distinct cavity opposite the fifth somite. The pronephric ducts extend backward to the anal region, and possess a lumen in their anterior portion. The pronephros is probably functional from this time to hatching.

The tail region is seem to be distinctly raised off the surface of the yolk, and to be rimmed in the median line by a continuous fin fold.

Developments Occurring Between Stages 228 and 400

Stage 272. O. S. U. Series.—(Incubation period, 46 days, 4 hours; 133.78 T. U.; 49 somites.) The liver appears for the first time in this stage. It is a ventral evagination from the mid-gut, at the level of the sixth somite.

Stage 288. O. S. U. Series.—(Incubation period, 48 days, 16 hours; 136.44 T. U.; 52 somites.) The pre-auditory or branchial sense organ placode is now separated from and independent of the auditory vesicle. The 3rd and 4th branchial pouches are present in succession behind the branchial cleft. Myofibrils have formed in the somites throughout the middle of the trunk. In this same region the dorsal aorta is conspicuous, but devoid of blood cells. Below it lies a large intermediate mass of cells which constitute blood islands. From this mass the axial vein and the erythrocytes of the embryonic blood supply become differentiated. The embryonic coelom is conspicuous also at this level. The gut is surrounded by splanchnopleural folds and it is suspended by a dorsal mesentery.

Stage 304. (Incubation period, 51 days, 12 hours; 139.1 T. U.; 56 somites.) At this stage the pectoral fins appear as lateral masses of undifferentiated mesenchymatous tissue, covered by a thin layer of squamous epithelium. They are small, rounded, lobate structures extending laterally from the body just posterior to the head region. Their faint outlines in surface views of whole mounts are seen at this stage with difficulty, but by Stage 400 they are clearly visible. They apparently arise as outgrowths from the body wall, with which they are closely associated, rather than from the head mesoblast as described by Wilson in *Serranus*.

Stage 320. (Incubation period, 54 days, 4 hours; 141.76 T. U.; 62 somites.) For the last several stages described, the cells in the anterior portion of the notochord have been undergoing vacuolation progressively caudad. As a result, the protoplasm of the notochordal cells in the anterior half of the embryo has been reduced to a thin peripheral layer. For the first time in the present stage, the notochord

is surrounded by a hyaline sheath which takes a deeper stain than the surrounding tissue. From this incipient condition, this hyaline cartilaginous sheath becomes increasingly thick and more rigid as development proceeds. Beneath the notochord, the dorsal aorta may be traced throughout the length of the body into the tail region, where the caudal vein runs beneath it. The subintestinal vein is visible beneath the intestine for the first time. The gut possesses a distinct lumen throughout its length. The splanchnopleural folds, which were seen to surround the mucosal layer of the primitive gut wall in Stage 288, have apparently split into a double layered condition. These two layers constitute the primordia of the muscular wall of the intestine and its peritoneal covering. Posteriorly, the gut penetrates the ventral fin-fold of the body wall and ends at its lower margin. The vent is virtually closed. A few sections in advance of it the pronephric ducts unite and enter the dorsal wall of the gut at a point which represents the roof of the cloaca.

The fin-fold around the tail is composed of a folded layer of epidermis. Interrupted at the vent, it continues forward as far as the body is free from the yolk.

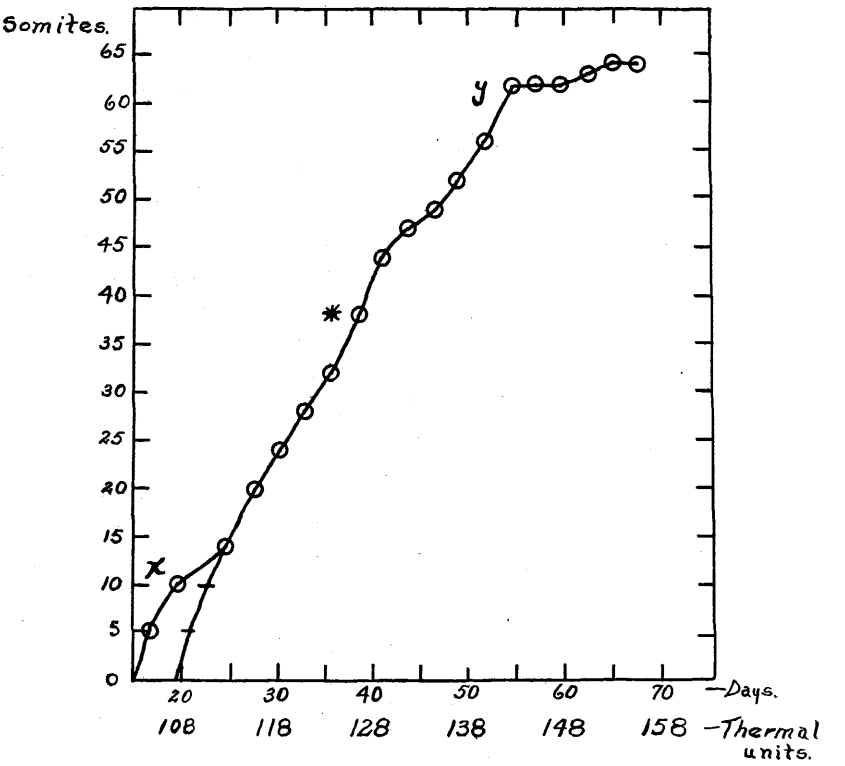
Stage 352. (Incubation period, 59 days, 12 hours; 147.08 T. U.; 62 somites.) The pronephric chambers on either side at the level of the 4th and 5th somites have become enlarged and have migrated farther than previously toward the midline. Their mesial surfaces are infolded and enclose short branches of the dorsal aorta to form a simple glomerulus. The chamber ends in a blind sac at the level of the sixth somite.

Stage 368. (Incubation period, 62 days, 4 hours; 149.74 T. U.; 64 somites.) With this stage, the full adult number of somites is present in the embryo. At no time in the remaining stages of this series has a greater number of somites been found. The number, then, of sixty-four somites may be regarded as the hatching number, and the definitive number for this species. It is of interest to note that the complete number of somites is differentiated in the whitefish before the embryo has completed the first half of its incubation period.

Once the formation of the paired somites is initiated, an increase in their number apparently occurs at a rate directly proportional to the number of thermal units to which the embryo has been subjected. In the foregoing descriptions, the length of the incubation period of each stage in this series is given in terms of both time and thermal units following fertilization. It is seen from these data that at Stage 112, no paired somites were present. By Stage 120, five somites were present and the sixth was partly formed. This embryo had undergone 105 thermal units of incubation.

The complete number of somites was acquired when the embryo had been subjected to an incubation period of 150 Thermal units. When the numbers of somites for the different stages through this part of the embryonic period are plotted on a graph as ordinants and the thermal units of incubation for these same stages are plotted as abscissae, the resulting values form practically a straight line curve. (See Graph No. 1.) This may be interpreted as expressing a direct

relationship between these two sets of factors, the number of somites increasing at a rate directly proportional to the sum of the time and temperatures to which the embryo has been exposed. The thermal units have been calculated from daily temperature and time charts of the incubation period, following the method described in the first paper of this series. If the thermal unit is a valid criterion for deter-



GRAPH No. 1. The relation between the age of the whitefish embryo, expressed in terms of days and thermal units of incubation, and the number of somites present. The small circles indicate thermal unit values. The symbol x indicates the period of initial somite formation; the asterisk, *, the stage when the heart and pronephric tubules becomes established; the symbol y, the appearance of definitive axial blood vessels.

mining comparable stages in different series of embryos, then embryos of the same age in number of thermal units will have a similar number of somites.

The curve is actually not straight but rather a wavy line. There are three periods in which the growth increment is greater than in intermediate ones. The first, indicated by the letter x, on the graph, is an initial period of rapid growth, when the first few somites are being laid down. Then the rate is gradually retarded up to about 125 Thermal

units of incubation. A second period (*) of more rapid growth is then initiated. It is of interest to note that at this time two significant events occur. The heart is formed as a distinct tube, but devoid of blood cells, and the pronephric ducts for the first time acquire their lumen, and extend to the anus. Both the circulatory and excretory systems probably begin to function at this time. The probability that these systems influence the growth rate of the somites is strengthened by the fact that in the amniotes the growth rate of the embryo is influenced by them in a similar manner (Schmalhausen, 1927). The third cresty in the curve occurs just after the dorsal aorta and the axial vein become distinguishable throughout the length of the embryo, and are filled with blood.

The curve now bends rapidly to the right. The formation of additional somites is here apparently greatly retarded by inhibiting factors which operate to prevent the formation of more than sixty-four somites in this species. The embryo hereafter possesses this same number of somites. The curve for these later stages then would be a straight line.

The author is unable to say just how typical this curve with its fluctuations is for the species as a whole. The number of embryos included in this study is too few to warrant the deduction of very definite conclusions. Several embryos for each stage must be examined to determine the degree of variation within each stage before the relationship between these various factors may be stated with any degree of certainty.

In the myotomal region of the anterior somites the myofibrils have a beaded appearance, due to the presence of alternate light and dark bands. These collectively, of course, give the appearance of cross-striations to these fibres.

Stage 400. (Incubation period, 67 days, 12 hours; total length of embryo, 8 mm.; 64 pairs of somites; 155.06 T. U.) (Reconstruction drawings, Plate IV.)

Despite the fact that the embryo at this stage has only completed one-half of its incubation period, it has now acquired two-thirds of its length at hatching (12 mm.) and the complete number of somites which it will possess at that time—sixty-four.

The embryo has been forced by the constant shrinking of the yolk sac to assume a curled position upon it. The tail which was becoming free from the yolk in Stage 224 (Plate III) has now become bent back on the body to form an almost complete circle on the surface of the yolk at this stage. This condition makes it impossible to reconstruct the embryo on a plane surface drawing in the coiled portions of the body. To do so would necessitate a drawing of one part of the coil seen behind or through the other, or viewed at an angle, which latter would introduce errors in perspective. It was found to be impossible to straighten the embryo from its curved position. Hence simply the anterior portion of the body, back to the first sharp curve, has been included in these drawings.

By a comparison of Stage 400 with Stage 224, it is seen that several noteworthy changes have occurred. During this interval the embryo has approximately doubled its total length, from 4.2 mm. to 8 mm. There has

been a great lateral growth of the body, especially in the head region. The depth of the head in the two stages is about the same; but seen from the dorsal surface (not shown in the drawings) the body and head, including the brain, have greatly expanded laterally. The medulla in Stage 400, side view, appears thinner and more shallow than in Stage 224. This is actually the case, but the thinning process is brought about by a lateral growth of the medulla which has approximately doubled its former width. The cavity of the fourth ventricle above it has also greatly expanded, and the membranous choroid plexus is a prominent landmark. There has been a correspondingly great lateral growth of the midbrain, and the differentiation of the metencephalon. The latter is now a thin transverse plate separated from the mesencephalon by a transverse fissure.

In Stage 224, the nasal pit is terminal on the forebrain. In Stage 400, there has been a downward and backward growth of the forebrain and the infundibulum, carrying the nasal pit with it until it comes to lie ventrally.

The expansion of the lobes of the brain, and their further differentiation into their definitive parts, has brought about a thinning of their walls, leaving cavities within. In Stage 224 no expanded cavities traceable in a drawing were present. As a result of the backward and under growth of the forebrain, the infundibulum is very prominent, and extends to a point in close proximity to the anterior end of the fore-gut. This growth has also carried the eye with it in its backward migration with the result that the eye and the ear are in much closer proximity to each other than formerly. It will be seen that the changed position must be due to the shifting of the forebrain, since the relative position of the ear to the medulla and to the anterior end of the notochord remains essentially the same as in Stage 224.

The foregut ends in the same relative position as in the latter stage. There is no visible connection seen between the foregut and the infundibulum, nor any semblance of a stomodaeum present.

The branchial pouches are broadly extended laterally. The hyomandibular pouch is more restricted in extent than in earlier stages. The first branchial cleft is the only one open at this stage. Branchial pouches II, III, IV, all lie posterior to the otic vesicle. They are separated from each other by an undifferentiated mass of head mesoderm which comprises the visceral arch. The pharynx has grown very broad here in comparison with the condition shown on the previous drawing, Stage 224, Plate III, in correlation with the lateral growth of the head. From the drawing of the median sagittal section, it is seen that there is no lumen present in the pharynx, and that it does not appear anterior to the gastric region, which is an expanded area at the level of the pectoral fin. Here the columnar epithelium is well differentiated. Surrounding this part of the gut, the liver is forming as an elongated glandular outgrowth closely applied to the rounded gut wall.

The tubular heart is considerably larger than in Stage 224. It has become elongated and now extends backward to the level of the esophagus, and bends in a U-shaped loop away from the median line. Only the two ends of the loop pass across the median line. The

heart is now suspended by the mesocardium within the pericardial cavity. The vitelline veins are large and are filled with erythrocytes. The short ventral aorta leads forward from the heart into the aortic arches which pass laterally over the pharyngeal folds.

The auditory vesicle is gradually undergoing differentiation. Its outline is no longer smoothly circular, but somewhat irregular, indicating three bulbous prominences, which will develop later into the ampullae at the bases of the semicircular canals.

The preauditory sensory placode was described in Stage 288 as having separated from the auditory vesicle but lying immediately anterior to it. Since that stage, this placode has apparently migrated forward. It now lies on the side of the head dorsal to the posterior curvature of the orbit and anterior to the level of the hyomandibular pouch. It is a single, elongated sensory patch. Its cells have become elongated and somewhat clustered.

The first anterior pair of somites lies immediately behind the auditory vesicle. These 64 pairs of somites are clearly seen in the whole mounts. Their cells are differentiated and contain striated myofibrils. Beginning with the fifth or sixth pair, each somite has been divided by a horizontal constriction into a dorsal and lateral portion. These muscle masses are probably capable of contraction to cause bodily movements.

The pronephric chambers each have a single glomerulus on their mesial border, and the pronephric tubules enter the dorsal portion of the cloaca as described in Stages 320 and 352.

The paired pectoral fins which were previously described as first appearing in Stage 304 are now definitely raised up from the surface of the lateral somatopleure and possess a median longitudinal fin fold. They are subtriangular in shape and lie opposite somites III to VII.

SUMMARY

This paper is the second of a series of three papers on the embryology of the whitefish. The author's purpose is to locate the incipient stages in the development of each of the organ systems and to trace their general development in this particular series of embryos.

It is seen from the descriptions of Stages embodied in this paper that every line of development in the whitefish embryo is well established by Stage 400, which marks the end of the first half of the incubation period of this series in terms of time and temperature under normal hatchery conditions.

Since the closure of the blastopore, differentiation and growth have converted the primitive embryo into one of distinct fish-like form. It is now completely coiled on the yolk, and is two-thirds its hatching length. The tri-lobed brain has developed its definitive regions, and the nose, eye and ear are advanced well beyond the vesicular condition.

The differentiation of the organs derived from the mesoderm scarcely begins before the closure of the blastopore. The growth of the notochord forward, the vacuolation of its cells, and its investment by a cartilaginous sheath has been traced. The increase in the number of somites to the full number, and the differentiation within the muscle masses of striated myofibrils has been described. The development

of the heart, axial blood vessels, and definitive blood cells has brought the circulatory system up to a stage where it is probably functional. The same is true of the pronephric kidneys which are seen to develop concurrently. The visceral arches, pericardium and the coelomic cavity are all well established.

The entoderm comprised an unfolded primary germ layer at the time the blastopore closed. Since that stage, the gut has become a closed tube throughout its length. The first of the branchial pouches has broken through the pharyngeal wall to form a cleft and the remaining three pouches are well defined. The liver and the lumen of the gastric region have developed. The anus has not ruptured through the ventral fin fold.

The primitive streak, so prominent when the blastopore closed, has gradually shortened, and its substance has contributed to the formation of structures in the tail region. Kupffer's vesicle, formerly so prominent, has disappeared in the region of the anus.

Thus the period of organogenesis closes. Before the embryo may emerge from its egg shell, growth and further differentiation must bring it to the hatching state. The outstanding phases in this later development of the whitefish will be discussed in the paper which is to follow.

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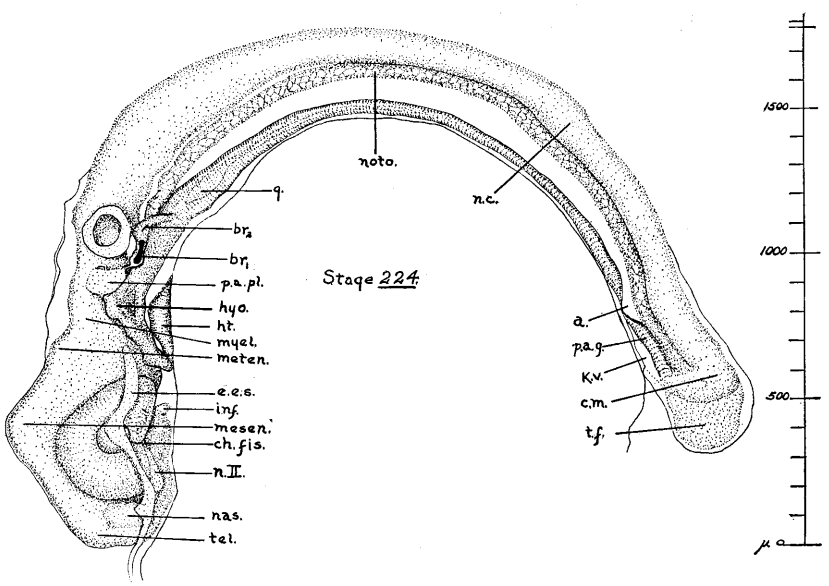
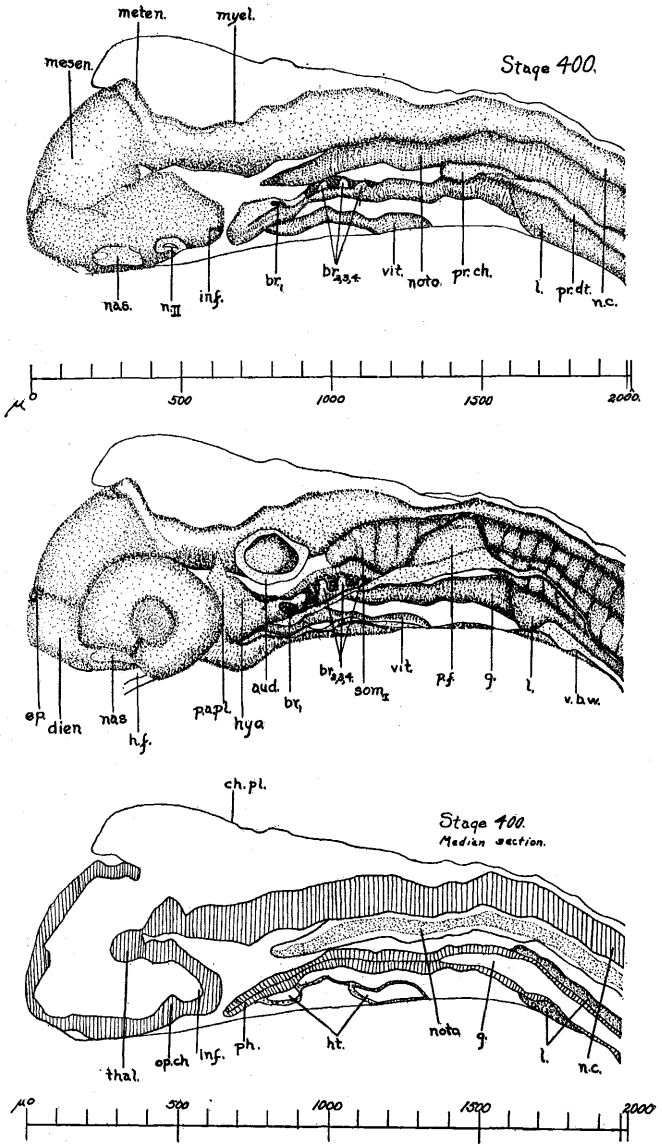
ABBREVIATIONS USED IN PLATES III AND IV

a.....anal region
aud.....auditory vesicle
br. 1.....first branchial cleft
br. 2, 3, 4..branchial pouches
ch. fis.....choroid fissure
ch. pl.....choroid plexus
c. m.....caudal mass, or primitive streak
dien.....diencephalon
e. e. s.....extra-embryonic somatopleure
ep.....epiphysis
g.....gut
h. f.....head fold
ht.....heart
hyo.....hyomandibular pouch
inf.....infundibulum
K. v.....Kupffer's vesicle
l.....liver
mesen.....mesencephalon
meten.....metencephalon
myel.....myelencephalon
n. II.....optic nerve
nas.....nasal pouch
n. c.....nerve cord
noto.....notochord
op. ch.....optic chiasma
p. a. g.....post-anal gut
p. f.....pectoral fin
ph.....pharynx
p. a. pl.....preauditory placode
pr. ch.....pronephric chamber
pr. dt.....pronephric duct
som. I.....first ant. somite
tel.....telencephalon
t. f.....tail fold
thal.....thalamus
v. b. w.....ventral body wall
vit.....vitelline vein



Reconstruction drawing of Stage 224, O. S. U. series, in natural position over the yolk. Length of embryo, 4.2 mm.; 39 somites.



Reconstruction drawings of Stage 400, O. S. U. series, anterior end of embryo. Embryo length, 8 mm.; 64 somites. This stage marks the end of the first half of the incubation period.

Fig. a (upper.) Reconstructed at the level of the brain surface, to the left of the median line.

Fig. b (center.) More lateral than Fig. a, showing sense organs, paired somites, pectoral fin, etc.

Fig. c (lower). Median sagittal section.

All three figures are drawn at the same magnification.